Oncogene (2000) 19, 1045–1051

www.nature.com/onc

Mutant p53 and genomic instability in a transgenic mouse model of breast cancer

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Oncogene (2000) **19,** 1045–1051.

Keywords: mutant p53; mammary tumorigenesis; DMBA; transgenic mice; review

Introduction

Tumorigenesis is a multistage process involving multiple genetic aberrations (Fearon and Vogelstein, 1990). These aberrations may include mutations in cellular proto-oncogenes that constitutively activate a growth signal transduction pathway, inactivation of tumor suppressor genes, and inactivation of genes that promote cell death. Other mutations provide an indirect growth advantage by compromising the genetic stability of the cell, increasing the occurrence of subsequent genetic lesions (Tlsty *et al*., 1995). Each successive mutation may then enhance the tumorigenic potential of the cell (Nowell, 1976). Advances in transgenic and knockout technologies make it possible to genetically engineer mice to mimic the individual steps in this process in order to better understand events contributing to cancer progression at the molecular level. Specifically, mouse models prone to genetic instability have been generated that may be useful to screen for early molecular events involved in carcinogenesis.

p53 is the most commonly mutated gene in human cancers, with approximately 40% of tumors displaying some alteration in p53 (Osborne *et al*., 1991). Unlike deletion or nonsense mutations observed in other tumor-suppressor genes, most p53 alterations are missense mutations resulting in the expression of a functionally altered protein (Hainaut *et al*., 1997). Wild-type p53 has been called the 'guardian of the genome,' as p53 responds to DNA damage or checkpoint failure by either arresting the cell in the G1 phase of the cell cycle for damage repair or through the initiation of an apoptotic pathway to eliminate the damaged cell entirely (Lane, 1992). Wild-type p53 is particularly critical for the maintenance of genomic stability; aberrant ploidy, gene amplification, increased recombination, and centrosomal dysregulation have been observed in cells lacking p53 (Donehower, 1997). Mutations in p53 may result not only in a loss of wild-type function, but also in the generation of dominant-negative and gain-of-function mutants (e.g., Dittmer *et al*. (1993)).

Specific p53 mutations, including those at codon 175, have been associated with a poor prognosis in breast cancer patients, and also with primary resistance to chemotherapy (Aas *et al*., 1996). This is one of five 'hotspot' codons present in the sequence-specific DNA binding domain of p53 that represent ~20% of all p53 mutations reported (Hainaut *et al*., 1997). Class II mutations, such as those at codon 175, affect residues crucial for maintenance of the correct orientation of the DNA-binding surface of non-contiguous loops and helices (Cho *et al*., 1994). Amino acid 175 is not located within the regions of the p53 protein that directly contact DNA, as are most of the other commonly mutated residues. The arginine side chain participates in bonds bridging loops 2 and 3 of the protein (Cho *et al*., 1994), and several lines of evidence suggest that the protein is at least partially unfolded as a result of the side chain substitution (Cho *et al*., 1994). The 175 R-H mutant human p53 protein is incapable of binding a consensus p53 DNA-binding site (Kern *et al*., 1991, 1992; Ory *et al*., 1994). The unique properties of certain p53 mutants may reflect their selective activation of specific DNA targets (Dittmer *et al*., 1993; Thukral *et al*., 1995) and/or participation in novel protein-protein interactions (Chen *et al*., 1994).

The development of both p53 knockout and p53 mutant transgenic mice has greatly facilitated studies of the role of p53 in carcinogenesis and tumor progression (Donehower, 1996). By crossing p53 null mice with lines of transgenic mice overexpressing specific oncogenes it has been possible to gain new insights into the mechanisms by which different signal transduction pathways interact with p53 to affect tumorigenesis (e.g., Donehower *et al*. (1995)). However, p53 knockout mice have some limitations for experiments designed to determine the role of p53 in mammary tumorigenesis, as these mice frequently die from lymphomas and sarcomas prior to mammary tumor development (Donehower *et al*., 1992). Consequently, mice containing a mutant p53 transgene targeted specifically to the mammary gland have been generated for these studies. The 175 R-H mutation is the second most-frequent p53 mutation observed in breast cancer, accounting for approximately 6% of those reported to date (Hainaut *et al*., 1997). In order to study the role of the murine-equivalent 172 R-H mutant p53 protein in mammary tumorigenesis, a genomic minigene construct containing this mutation (Li *et al*., 1998) was targeted specifically to the mammary gland of transgenic mice using the whey acidic protein (WAP) promoter (Bayna and Rosen, 1990).

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The WAP-p53 172 R-H transgenic model

The 172 R-H transgenic mice exhibit a negligible level of spontaneous tumorigenesis (Li *et al*., 1997, 1998). Transgene expression alone does not alter normal mammary development at the gross histological level, and as assessed through the analysis of apoptosis during involution and proliferation during pregnancy (Li *et al*., 1998). The mutant protein also does not alter the expression levels of p21, MDM2, proliferative cell nuclear antigen (PCNA), or several other genes known to be regulated directly by wild-type p53 at the transcriptional level (Li *et al*., 1998). To determine whether the presence of the transgene predisposed these mice to mammary tumorigenesis, they were given pituitary isografts to stimulate transgene expression (Medina, 1974) and treated with the carcinogen, dimethylbenz(a)anthracene (DMBA). Tumors arising in carcinogen-treated nontransgenic (FVB) and transgenic mice were analysed to determine the mechanisms by which this mutant p53 might promote tumorigenesis in the mammary gland.

Carcinogen susceptibility and tumor analysis

The 172 R-H transgenic mice developed tumors significantly more rapidly than controls, and exhibited a greater tumor burden (Li *et al*., 1998). One hundred per cent of transgenic mice developed tumors by week 28 post-DMBA treatment, while only 85% of FVB nontransgenic mice developed tumors by week 45 (Li *et al*., 1998).

Apoptosis and cell proliferation in tumors from transgenic and nontransgenic mice were compared, but no significant differences were found (Li *et al*., 1998). Loss of p53 function has been shown in the choroid plexus to influence tumorigenesis primarily through the inhibition of apoptosis (Symonds *et al*., 1994), but this does not appear to be the case in the mammary gland (Jones *et al*., 1997). However, tumor cell nuclei from the transgenic mice (Figure 1a) were in most cases larger and more pleomorphic than those from tumors arising in nontransgenic mice. Since p53 loss (Cross *et al*., 1995; Fukasawa *et al*., 1996) or mutation (e.g., Liu *et al*. (1996)) has been shown to result in genomic instability, the DNA content of populations of tumor cells from transgenic and nontransgenic mice was assessed by flow cytometry. Aberrant ploidy was more often seen in carcinogen-induced tumors from transgenic animals (Figure 1b) than in carcinogen-induced tumors from control animals (Li *et al*., 1998).

Bitransgenic model systems

Mice carrying the 172 R-H transgene have also been crossed in separate experiments with mice overexpressing MMTV-neu (*erb-B2*) (Li *et al*., 1997), WAP-*des*-IGF-1 (Hadsell *et al*., 1999), or WAP-TGF-α (K Murphy and J Rosen, 1999, unpublished results). Co-expression of MMTV-*neu* or WAP-*des*-IGF-1 with WAP-p53 172 R-H significantly decreased tumor latency relative to that seen with the oncogene alone (Hadsell *et al*., 1999; Li *et al*., 1997). In the WAP-TGF-α cross, both the bitransgenic and single transgenic TGF-α mice developed tumors with a mean latency of approximately 100 days (Figure 2), and because of this short latency no significant difference between the two groups was observed. Mammary tumors from mice expressing the p53 172 R-H transgene in conjunction with any of these growth factors or receptors were frequently aneuploid (Figure 1f), as assessed by flow cytometry, but tumors from mice expressing only the *neu*, *des*-IGF-1, or TGF-α transgene were not (Figure 1d). Tumors from bitransgenic mice also contained large irregular nuclei (Figure 1e) similar to those seen in tumors from the DMBA-treated transgenic mice (Hadsell *et al*., 2000, submitted; Li *et al*., 1997; and K Murphy and J Rosen, 1999, unpublished results).

These results suggest that the p53 172 R-H transgene predisposes female mice to the development of aneuploid mammary tumors once some other initiating event (i.e., oncogene co-expression in the mammary gland or carcinogen treatment) has taken place. Since the expression of the p53 172 R-H transgene alone resulted in very few spontaneous tumors in mice less than a year old (Hadsell *et al*., 2000, submitted; Li *et al*., 1997, 1998), while accelerating tumorigenesis caused by both carcinogen treatment and oncogene expression, this appears to be an excellent model system in which to study early events in mammary tumorigenesis. Furthermore, although most advanced-stage human breast cancers are aneuploid, mammary tumors generated in most mouse model systems to date have been uniformly diploid, which limits the utility of these models. This is one of the few model systems that consistently generates aneuploid tumors similar to grade 3, high S phase, hormone-independent human breast cancers. Patients with these types of tumors usually have the poorest prognosis. Other model systems generating aneuploid mammary tumors include p53-deficient mammary gland (Jerry, this volume), p53-deficient *Wnt-1* transgenic mice, which develop tumors exhibiting recurring changes on several chromosomes (Donehower *et al*., 1995), MMTV-*neu* mice, which frequently develop mammary tumors exhibiting loss of heterozygosity on chromosome 4 (Ritland *et al*., 1997), C3-driven SV40 Tag transgenic mice, which develop mammary tumors consistently showing DNA gains on chromosome 6, and WAP-Str1 (stromelysin-1) mice, which develop mammary lesions containing consistent genomic changes (Sternlicht *et al*., 1999).

Interestingly, although the WAP-TGF-α transgene is a potent mammary oncogene (expression results in short mammary tumor latency), when these mice are crossed with mice carrying another codon 172 p53 mutant generated in our laboratory, WAP-p53 172 R-L (Figure 2), mammary tumorigenesis is almost completely prevented (K Murphy and J Rosen, 1999, unpublished observations). When the p53 172 R-L mice and control nontransgenics were treated with the carcinogen DMBA, mammary tumorigenesis was delayed in the transgenic mice because of high levels of mammary epithelial cell apoptosis (~20%) induced by this transgene, which retains many properties of wild-type p53 (Li *et al*., 1995). Presumably the p53 172 R-L transgene is blocking tumorigenesis in the WAP-p53 172 R-L/WAP-TGF-α bitransgenic mice by a similar mechanism. The difference in tumorigenic properties between the 172 R-H and 172 R-L p53 mutants is striking, given that they occur at the same codon and are both found in human breast tumors.

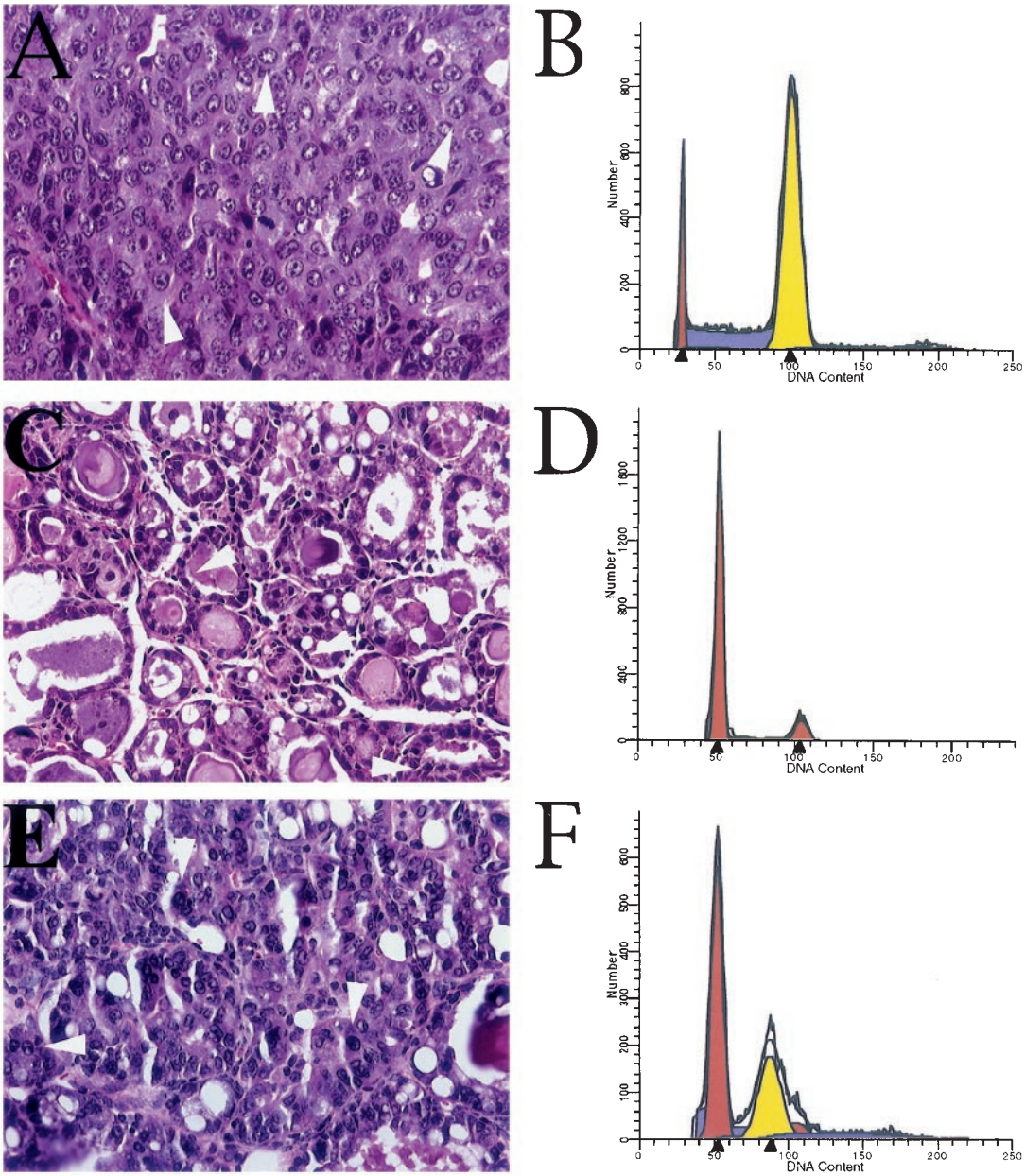

Figure 1 Histology and ploidy analysis of transgenic mouse tumors. (**a**), (**c**) and (**e**) Hematoxylin-and-eosin stained sections of tumors arising in representative WAP-p53 172 R-H (spontaneous), WAP-TGF-α, and WAP p53 172 R-H/WAP-TGF-α bitransgenic mice respectively, while (**b**), (**d**) and (**f**) represent flow cytometric analyses of the same tumors. The white arrowheads in (**a**) and (**e**) indicate representative large irregular nuclei, while the arrowheads in (**c**) point to normal-sized nuclei for comparison. The yellow peaks in (**b**) and (**f**) indicate the presence of aneuploid populations of cells in the WAP-p53 172 R-H and WAP-p53 172 R-H/WAP-TGF-α tumors

p53 172 R-H as a gain-of-function mutant

The 172 R-H p53 protein is a dominant-negative mutant in that it can interact with wild-type p53, but is no longer capable of specific DNA binding (Kern *et al*., 1991, 1992; Ory *et al*., 1994). This mutant, therefore, loses many of the direct transcriptional regulatory capabilities of wild-type p53. However, it appears to confer novel functions, indicating that it is a gain-of-function mutant. For example, it is capable of stimulating expression of MDR-CAT (a human multi-drug resistance {MDR}-1 gene promoter-CAT construct) in p53-null cells, in a manner reversible by co-transfection of wild-type p53 (Chin *et al*., 1992). When the 175 R-H mutant was transfected into p53-null Saos-2 cells, it conferred a growth advantage. Injection of a cell line expressing this mutant p53 protein into nude mice resulted in tumorigenesis, which was not seen with the parental p53-null cells (Dittmer *et al*., 1993). This mutant protein was also able to cooperate in co-transfection experiments with activated H-*ras* in the transformation of rat embryo fibroblasts (Hinds *et al*., 1990). Furthermore, cells containing this p53 mutation exhibit a dominant gain-of-function defect in spindle (G2/M) checkpoint control. When incubated with colcemid, a spindle assembly inhibitor, cells containing wild-type p53 arrest with 4n DNA content, but cells containing this p53 mutant can re-enter S phase and subsequently become polyploid (Gualberto *et al*., 1998). p53 has been implicated in the regulation of the G2/M spindle checkpoint and mitosis (Cross *et al*., 1995; Fukasawa *et al*., 1996;

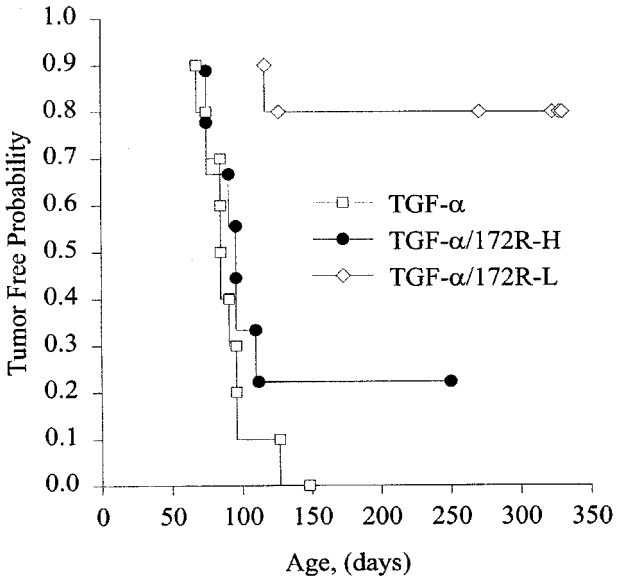

Figure 2 Tumor latency in pituitary isografted WAP-TGF-α, WAP-TGF-α/WAP-p53 172 R-H, and WAP-TGF-α/WAP-172 R-L female mice. The rapid (~100 days) mammary tumorigenesis induced by the WAP-TGF-α transgene is not accelerated by the WAP-p53 172 R-H transgene in the WAP-TGF-α/WAP-p53 172 R-H bitransgenic females. However, the WAP-p53 172 R-L transgene almost completely eliminates mammary tumorigenesis in the WAP-TGF-α/WAP-p53 172 R-L bitransgenic females, indicating that the latter p53 transgene has a protective effect

Gualberto *et al*., 1998; Paulovich *et al*., 1997; Stewart *et al*., 1995), with particularly striking effects upon centrosomal duplication (Fukasawa *et al*., 1996).

Primary skin tumors from mice bearing a skin-targeted p53 172 R-H mutation (murine amino acid 172 is equivalent to human 175) display a much greater degree of aberrant centrosomal duplication than do tumors from p53-null mice (Wang *et al*., 1998). Centrosomal amplification is implicated in at least two processes that adversely affect prognosis in cancer patients: (1) loss of cell polarity and tissue organization, and (2) an increased occurrence of multipolar mitoses, which predisposes to the development of aneuploidy, as it promotes unequal division of genetic material (Lingle *et al*., 1998). A recent study found that centrosomes in high-grade breast adenocarcinoma cells are larger and more numerous and contain more centrioles and pericentriolar material than do normal breast specimens. They are also inappropriately phosphorylated, and nucleate abnormally large numbers of microtubules (Lingle *et al*., 1998).

Approximately 50% of mammary tumors arising in the WAP-172 R-H/WAP-TGF-α bitransgenics in our experiments were aneuploid, while aberrant ploidy was not seen in any of the tumors arising in similarly treated WAP-TGF-α single transgenic females. As both p53 loss and p53 mutation have been associated with centrosome dysregulation and aneuploidy, centrosome numbers in thick frozen sections from both groups of mammary tumors were assessed by confocal microscopy using standard (fluorescent) immunohistochemical techniques. Surprisingly, neither group of tumors demonstrated centrosome abnormalities above background levels (with 'background' defined as $\leqslant 10\%$ by BR Brinkley, personal communication), despite the differences in tumor ploidy (K Murphy, BS Kolle, T Goepfert, J Zhong, BR Brinkley and J Rosen, 1999, unpublished observations).

In order to eliminate the possibility that centrosome dysregulation was occurring in the bitransgenic females in early stages of mammary tumorigenesis (i.e., before frank tumors were discovered) and promoting the later development of aneuploid tumors in that manner, a study of precancerous mammary glands was performed in this system. As discussed above, both the bitransgenic WAP-p53 172 R-H/WAP-TGF-α and the single-transgenic WAP-TGF-α females develop mammary tumors within approximately 100 days following the surgical implantation of a pituitary isograft to stimulate transgene expression, providing a reasonably short window in which to look for early centrosomal dysregulation induced by the p53 172 R-H transgene. Isografts were given to additional groups of young bitransgenic and WAP-TGF-α females, and also to single transgenic WAP-p53 172 R-H and nontransgenic (FVB) females for comparison. Mammary glands were surgically excised from these groups of mice at defined timepoints following isografting (15d, 30d, 45d, 60d, and 90d) and analysed as above for centrosome amplification. The histology of some of these lesions can be found at http://mammary.nih.gov/cgi-bin/imaged_b/output.taf. Again, no abnormal centrosome numbers were observed in any of the four groups of mice, at any timepoint (K Murphy and J Rosen, 1999, unpublished observations). These studies suggest that while centrosome dysregulation is known to promote genomic instability and tumorigenesis, it is not a prerequisite for the development of aneuploid tumors in the mouse mammary gland. However, we cannot formally exclude the possibility that aberrant centrosome duplication leading to genetic instability in a subpopulation of cells may be occurring before the 15-day timepoint in our studies. Most cells with an aberrant number of centrosomes would be predicted to undergo apoptosis following multipolar cell division, but a few may survive, giving rise to a potentially pretumorigenic subpopulation of cells that might not have been detected in these assays.

Currently, there is much debate as to the precise relationship between aberrant centrosome duplication and aneuploidy. Our results with the p53 172 R-H transgenic mice support the hypothesis that centrosome amplification does not necessarily precede the development of aneuploidy. This is further supported by a study of aneuploid mouse mammary tumors that arise in hormonally-treated Balb/c mice that have had syngeneic p53-null mammary epithelial cells transplanted into their cleared fat pads, which showed that very few of these tumors had aberrant numbers of centrosomes (D Medina, personal communication). Conversely, in a NMU-induced rat mammary tumor system, aberrant centrosome numbers are seen, but the tumors are near-diploid (BR Brinkley, personal communication). In contrast, when MCF7 (BR Brinkley, personal communication) and MCF10A (Zhou *et al*., 1998) cells are transfected with the centrosome-associated kinase BTAK, centrosome amplification and aneuploidy are seen concurrently. At present, it appears that centrosome amplification may neither be necessary nor sufficient to induce aneuploidy, but that it does constitute one mechanism by which aneuploidy may be initiated.

Recently it has been suggested that at least some genomic instability is the result of failures in the DNA repair pathway. Wild-type p53 has been reported to interact with BRCA1 (Chai *et al*., 1999; Zhang *et al*., 1998), BRCA2, and RAD51 (Marmorstein *et al*., 1998; Sharan *et al*., 1997). Thus, this multiprotein complex (Figure 3) may play an important role in DNA repair (Chen *et al*., 1999; Patel *et al*., 1998; Zhang *et al*., 1998). BRCA1 is also important for the cellular responses to DNA damage that are mediated through the hRad50-hMre11-p95 complex (Zhong *et al*., 1999). BRCA1 is known to physically associate with components of the RNA polymerase II general transcriptional apparatus, suggesting a role in transcriptional control and DNA repair (Chen *et al*., 1999) especially as BRCA1 is accompanied by Rad51 when it relocates to PCNA-positive replication sites following hydroxyurea or low-dose UV treatment of cells (Chen *et al*., 1999). Rad51 mutants fail to correctly repair double-stranded DNA breaks (Shinohara *et al*., 1992). BRCA1 is also required for transcription-coupled repair of oxidative DNA damage (Gowen *et al*., 1999). Fibroblasts derived from embryos carrying a targeted exon 11 BRCA1 deletion have a defective G2/M checkpoint, which is accompanied by extensive chromosomal abnormalities. They also contain multiple functional centrosomes, leading to unequal chromosome segregation and aneuploidy (Xu *et al*., 1999). BRCA1 is known to associate with the centrosome during mitosis (Hsu and White, 1998). Tumors carrying mutations in BRCA2 also show complex chromosomal changes (Gretarsdottir *et al*., 1998; Patel *et al*., 1998).

Wild-type p53 may itself play a direct role in DNA repair. It is known to preferentially bind free DNA ends, single-stranded DNA, short mismatched loops and radiation damaged DNA, and it can reanneal DNA strands (Donehower, 1997). It can also bind DNA-repair associated proteins such as ERCC3, RPA, XPB, and XPD and colocalize with them to sites of DNA repair (Donehower, 1997). It is conceivable that the 172 R-H mutant p53 protein may, therefore, be promoting genomic instability at least partially through disrupting the normal function of DNA repair complexes (Figure 3). It has also been suggested that mitotic checkpoint inactivation (such as that induced by p53 175 R-H [Gualberto *et al*., 1998]) may cooperate with BRCA2 deficiency to promote tumorigenesis in humans (Lee *et al*., 1999), lending further support to the hypothesis that disrupted interactions between members of the BRCA1/BRCA2/Rad51/p53 complex may be integrally involved in mammary tumorigenesis.

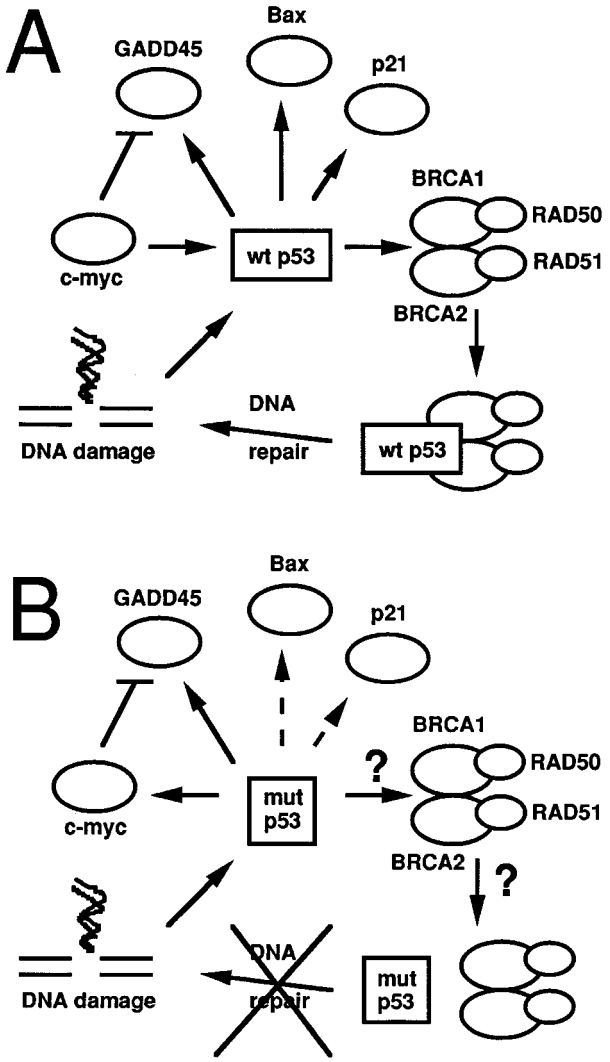

Figure 3 Differential functions of wild-type and mutant p53. (**a**) Depicts transactivational and protein-protein interactions made by wild-type p53, while (**b**) depicts the effect of the p53 172 R-H mutant p53 on the same interactions. Dotted lines in (**b**) indicate transactivational interactions that are no longer functional. We hypothesize that the wild-type p53 protein contributes to DNA repair after damage through its interaction with the BRCA1/BRCA2/Rad51 complex, an interaction that may not be functional when there are mutations in the p53 protein

Potential gain-of-function mechanisms

As p53 is a multifunctional protein, the p53 gain-of-function mutants may lose the ability to regulate transcription of certain target genes involved in cell cycle control and apoptosis, like p21 or Bax, that require DNA binding, but still retain other functions that require protein-protein interactions (Figure 3). The latter may fall into several categories. First, interactions with other transcription factors or co-activators could lead to transcriptional activation from novel promoters. It has recently been reported that p53 participates in transcriptional induction of the *GADD45* gene through an interaction with WT-1 bound to an Egr-1 site on the GADD45 promoter, but not as a result of direct DNA binding by p53 (Zhan *et al*., 1998). Second, nonsequence-specific interactions of the p53 carboxy-terminus with single-stranded DNA or RNA could affect gene regulation. For example, it has been reported that p53 mutants can induce c-*myc* gene expression through an interaction between the carboxy-terminal region of p53, which possesses a single-stranded DNA and RNA binding activity, and a region located at the exon 1/intron 1 boundary of c-*myc* (Frazier *et al*., 1998). This interaction may overcome the block to transcriptional elongation known to occur in the c-*myc* gene. Finally, nontranscriptional interactions such as those already known to exist between wild-type p53 and centrosome elements/microtubules (e.g., Brown *et al*. (1994)) could affect mitotic fidelity and genomic stability in early

tumor development. These mechanisms may account for the apparent 'gain-of-function' and predisposition to genomic instability that have been observed not only in transgenic mice overexpressing WAP-172 R-H p53, but also in cell culture systems.

Conclusions

Cancer initiation and progression are complex processes involving many genetic and epigenetic factors. One of the future goals of the National Cancer Institute is the development of improved mouse models to help elucidate the mechanisms underlying these processes and for use in testing new diagnostic and therapeutic regimens. In this regard, the WAP-p53 172 R-H transgenic model developed in our laboratory is unique in that it consistently produces tumors characteristic of high-grade breast adenocarcinomas. This model should, therefore, serve as an excellent system in which to study the mechanisms responsible for genetic instability and may help identify those factors that promote tumor progression and metastasis. Finally, because mammary gland abnormalities are rarely observed in this model in the absence of carcinogen administration or oncogene co-expression, this model should facilitate the identification of earlier genetic lesions.

Acknowledgements
This research was supported by grant CA 16303 from the National Institutes of Health. KL Murphy was supported in part by Cell and Molecular Biology Interdisciplinary Program training grant GM08231. The authors would like to thank Dr Rodolfo Laucirica for help with FACS analysis and Drs Daniel Medina, Darryl Hadsell, and Bill Brinkley for valuable discussions. We would further like to thank Dr Thea Goepfert, Jeannie Zhong, and Frank Herbert for help with centrosome staining and confocal microscopy.

References

Aas T, Børresen A-L, Geisler S, Smith-Sørenson B, Johnsen H, Varhaug JE, Akslen LA and Lønning PE. (1996). *Nature Med.,* **2,** 811–814.
Bayna EM and Rosen JM. (1990). *Nucl. Acids Res.,* **18,** 2977–2985.
Brown CR, Doxsey SJ, White E and Welch WJ. (1994). *J. Cell Physiol.,* **160,** 47–60.
Chai YL, Cui J, Shao N, Shyam E, Reddy P and Rao VN. (1999). *Oncogene,* **18,** 263–268.
Chen J-J, Silver D, Cantor S, Livingston DM and Scully R. (1999). *Cancer Research,* **59,** 1752S–1756S.
Chen Y, Chen PL and Lee WH. (1994). *Mol. Cell Biol.,* **14,** 6764–6772.
Chin K, Ueda K, Pastan I and Gottesman MM. (1992). *Science,* **255,** 459–462.
Cho Y, Gorina S, Jeffrey PD and Pavletich NP. (1994). *Science,* **265,** 346–355.
Cross SM, Sanchez CA, Morgan CA, Schimke MK, Ramel S, Idzerda RL, Raskind W and Reid BJ. (1995). *Science,* **267,** 1353–1356.
Dittmer D, Pati S, Zambetti G, Chu S, Teresky AK, Moore M, Finlay C and Levine AJ. (1993). *Nature Genetics,* **4,** 42–46.
Donehower L. (1996). *Biochim. Biophys. Acta,* **1242,** 171–176.
Donehower LA. (1997). *Cancer Sur.,* **29,** 329–351.
Donehower LA, Godley L, Aldaz CM, Pyle R, Shi Y-P, Pinkel D, Gray J, Bradley A, Medina D and Varmus HE. (1995). *Genes Dev.,* **9,** 882–895.
Donehower LA, Harvey M, Slagle BL, MacArthur MJ, Montgomery Jr CA, Butel JS and Bradley A. (1992). *Nature,* **356,** 215–221.
Fearon ER and Vogelstein B. (1990). *Cell,* **61,** 759–767.
Frazier MW, He X, Wang J, Gu Z, Cleveland JL and Zambetti GP. (1998). *Mol. Cell Biol.,* **18,** 3735–3743.
Fukasawa K, Choi T, Kuriyama R, Rulong S and Vande Woude GF. (1996). *Science,* **271,** 1744–1747.
Gowen LC, Avrutskaya AV, Latour AM, Koller BH and Leadon SA. (1999). *Science,* **281,** 1009–1012.
Gretarsdottir S, Thorlacius S, Valgardsdottir R, Gudlaugsdottir S, Sigurdsson S, Steinarsdottir M, Gunnlaugur Jonasson J, Anamthawat-Jonsson K and Eyfjord JE. (1998). *Cancer Res.,* **58,** 859–862.
Gualberto A, Aldape K, Kozakiewicz K and Tlsty T. (1998). *Proc. Natl. Acad. Sci. USA,* **95,** 5166–5171.
Hainaut P, Soussi T, Shomer B, Hollstein M, Greenblatt M, Hovig E, Harris CC and Montesano R. (1997). *Nucleic Acids Res.,* **25,** 151–157.
Hinds PW, Finlay CA, Quartin RS, Baker SJ, Fearon ER, Vogelstein B and Levine AJ. (1990). *Cell Growth Differ.,* **1,** 571–580.
Hsu L-C and White RL. (1998). *Proc. Natl. Acad. Sci. USA,* **95,** 12983–12988.
Jones JM, Attardi L, Godley LA, Laucirica R, Medina D, Jacks T, Varmus HE and Donehower LA. (1997). *Cell Growth Differ.,* **8,** 829–838.
Kern SE, Kinzler KW, Bruskin A, Jarosz D, Friedman P, Prives C and Vogelstein B. (1991). *Science,* **252,** 1708–1711.
Kern SE, Pietenpol JA, Thiagalingam S, Seymour A, Kinzler KW and Vogelstein B. (1992). *Science,* **256,** 827–830.
Lane DP. (1992). *Nature,* **358,** 15–16.
Lee H, Trainer AH, Friedman LS, Thistlethwaite FC, Evans MJ, Ponder BAJ and Venkitaraman AR. (1999). *Mol. Cell,* **4,** 1–10.
Li B, Kittrell FS, Medina D and Rosen JM. (1995). *Mol. Carcinog.,* **14,** 75–83.
Li B, Murphy KL, Laucirica R, Kittrell F, Medina D and Rosen JM. (1998). *Oncogene,* **16,** 997–1007.
Li B, Rosen JM, McMenamin-Belano J, Muller WJ and Perkins AS. (1997). *Mol. Cell Biol.,* **17,** 3155–3163.
Lingle WL, Lutz WH, Ingle JN, Maihle NJ and Salisbury JL. (1998). *Proc. Natl. Acad. Sci. USA,* **95,** 2950–2955.
Liu PK, Kraus E, Wu TA, Strong LC and Tainsky MA. (1996). *Oncogene,* **12,** 2267–2278.
Marmorstein LY, Ouchi T and Aaronson SA. (1998). *Proc. Natl. Acad. Sci. USA,* **95,** 13869–13874.
Medina D. (1974). *J. Natl. Cancer Inst.,* **53,** 213–217.
Nowell PC. (1976). *Science,* **194,** 23–28.
Ory K, Legros Y, Auguin C and Soussi T. (1994). *EMBO J.,* **13,** 3496–3504.
Osborne RJ, Merlo GR, Mitsudomi T, Venesio T, Liscia DS, Cappa APM, Chiba I, Takahashi T, Nau MM, Callahan R and Minna JD. (1991). *Cancer Res.,* **51,** 6194–6198.
Patel KJ, Yu VPCC, Lee H, Corcoran A, Thistlethwaite F, Evans MJ, Colledge WH, Friedman LS, Ponder BAJ and Venkitaraman AR. (1998). *Mol. Cell,* **1,** 347–357.
Paulovich AG, Toczyski DP and Hartwell LH. (1997). *Cell,* **88,** 315–321.

Ritland SR, Rowse GJ, Chang Y and Gendler SJ. (1997). *Cancer Research,* **57,** 3520–3525.

Sharan SK, Morimatsu M, Albrecht U, Lim D, Regel E, Dinh C, Sands A, Eichele G, Hasty P and Bradley A. (1997). *Nature,* **386,** 804–810.

Shinohara A, Ogawa H and Ogawa T. (1992). *Cell,* **69,** 457–470.

Sternlicht MD, Lochter A, Sympson CJ, Huey B, Rougier J-P, Gray JW, Pinkel D, Bissell MB and Werb Z. (1999). *Cell,* **98,** 137–146.

Stewart N, Hicks GG, Paraskevas F and Mowat M. (1995). *Oncogene,* **10,** 109–115.

Symonds H, Krall L, Remington L, Saenz-Robies M, Lowe S, Jacks T and Van Dyke T. (1994). *Cell,* **78,** 703–713.

Thukral SK, Lu Y, Blain GC, Harvey TS and Jacobsen VL. (1995). *Mol. Cell Biol.,* **15,** 5196–5202.

Tlsty TD, Briot A, Gualberto A, Hall I, Hess S, Hixon M, Kuppuswamy D, Romanov S, Sage M and White A. (1995). *Mutat. Res.,* **337,** 1–7.

Wang X-J, Greenhalgh DA, Jiang A, He D, Zhong L, Medina D, Brinkley BR and Roop DR. (1998). *Oncogene,* **17,** 35–45.

Xu X, Weaver Z, Linke SP, Li C, Gotay J, Wang X-W, Harris CC, Ried TC and Deng C-X. (1999). *Molecular Cell,* **3,** 389–395.

Zhan Q, Chen I-T, Antinore MA and Fornace Jr AJ. (1998). *Mol. Cell Biol.,* **18,** 2768–2778.

Zhang H, Somasundaram K, Peng Y, Tian H, Zhang H, Bi D, Weber BK and El-Deiry WS. (1998). *Oncogene,* **16,** 1713–1721.

Zhong Q, Chen C-F, Li S, Chen Y, Wang C-C, Xiao J, Chen P-L, Sharp ZD and Lee W-H. (1999). *Science,* **285,** 747–750.

Zhou H, Kuang J, Zhong L, Kuo W-L, Gray JW, Sahin A, Brinkley BR and Sen S. (1998). *Nature Genetics,* **20,** 189–193.